OPINION

# High-content analysis in neuroscience

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Abstract | High-content analysis (HCA) combines automated microscopy and automated image analysis to quantify complex cellular anatomy and biochemistry objectively, accurately and quickly. High-content assays that are applicable to neuroscience include those that can quantify various aspects of dendritic trees, protein aggregation, transcription factor translocation, neurotransmitter receptor internalization, neuron and synapse number, cell migration, proliferation and apoptosis. The data that are generated by HCA are rich and multiplexed. HCA thus provides a powerful high-throughput tool for neuroscientists.

Modern neuroscience research uses many advanced imaging methods. A particularly exciting recent development in the imaging and analysis field is that of high-content analysis (HCA). HCA is the process of extracting high-level information (by quantifying complex attributes) from images of cells and tissues using a combination of automated microscopy and automated image analysis (BOX 1; FIG. 1). In this Review I use the term HCA throughout and include high-content screening under this heading.

HCA is now used widely by those carrying out drug discovery, and there are a number of excellent reviews of the use of this technology in this area[1–6]. Although HCA has historically been used mainly by the pharmaceutical and biotechnology industries, it is also particularly suited to neuroscience research applications. Here I discuss HCA's potential as a tool for multiple applications in neuroscience research, in the hope of encouraging more academic neuroscience researchers to consider adopting HCA in their research. I describe what HCA is, discuss how it is carried out, compare it with manual methods of image analysis, provide examples of its application in neuroscience and discuss current limitations and future trends. Finally, I describe my own experiences in establishing and running an academic HCA facility.

## What is HCA?

Traditionally HCA was applied to cells that had been grown *in vitro* (cell-based assays), and a number of commercial inverted-automated-microscopy and image-analysis platforms that provide HCA capabilities for cells grown in microplates are available (including confocal and standard widefield platforms)[1]. These include, but are not limited to, Molecular Devices' Image Xpress$^{MICRO}$ and confocal ImageXpress$^{ULTRA}$, Cellomics' ArrayScan HCS Reader, GE Healthcare's IN Cell Analyzer, the Opera LX, and BD Pathway's Bioimager. Some of these systems can also image live cells and handle liquids, making them ideal for kinetic studies.

HCA can also be applied to images of sections of brain tissue, making it applicable to both *in vivo* and *in vitro* neuroscience. For example, HCA was recently used to identify and count neural progenitors in sections of the adult human brain, identifying for the first time the human rostral migratory stream[7]. In this study the image analysis was performed at high throughput, but the images were acquired using standard manual microscopic methods. The combination of HCA with brain tissue microarrays will allow greater standardization of image-acquisition parameters (such as exposure time and fluorescence intensity, which will reduce image variation between different brain sections) and higher throughput. HCA can also be applied to model systems, such as *Drosophila melanogaster*, the zebrafish and *Caenorhabditis elegans*: whole animals can be imaged and analysed at high throughput, and many phenotypic changes can be detected[8]. Additionally, brain-slice cultures could also be studied with HCA.

The key to HCA is automation of both image acquisition and image analysis[9]. Indeed, the high throughput in image acquisition by automated microscopy is one of the great advantages of HCA, because it allows large numbers of images to be acquired and thereby reduces the impact of experimental errors and biological variation. Thus, subtle changes that might usually be buried in noisy backgrounds, but that might be important from a physiological or pathological perspective, can be detected.

One of the most important considerations when using automated microscopes is how to optimize both image quality and acquisition speed (BOX 1). Equally important are the automated image-processing and analysis tools that 'clean up' images and extract the relevant information (BOX 1). The validity of any assay tool is important, and it is particularly important that HCA assays are validated against other methods, including manual human analysis and, where possible, biochemical assays. A trade-off between assay speed and accuracy is inevitable, and how these attributes are balanced depends on the purpose of the assay. For example, assays that are designed to indicate trends might trade off some accuracy for speed, whereas those that require precise outputs might sacrifice speed for accuracy. Clearly the ultimate goal is to maximize speed and accuracy (and thereby reduce both false positives and false negatives), as well as validity. For both neuroscience and biotechnology applications, high-resolution images provide accurate information and high-quality data and are therefore preferred. External terabyte-capacity hard drives have largely solved the storage issues that are associated with acquiring large numbers of high-resolution images.

### What are the advantages of HCA?

In essence HCA performs the same task that the human visual system performs, but it does so objectively, in a standardized fashion (that is, the same parameters are applied to all images) and quickly, greatly increasing the efficiency and consistency of the analysis. Here I list several real advantages of HCA image processing over manual methods of analysis.

***Standardization and objectivity.*** When comparing different conditions, the images must be acquired in a standardized way if meaningful, reliable and robust conclusions are to be drawn. In particular, when acquiring fluorescent images the exposure time and number of exposures must be controlled and standardized. Hence, automated microscopy platforms have a big advantage over manual platforms. Furthermore, although a human element is required to set the optimal parameters for segmenting and measuring objects in images, once these are set they can be applied to all images in a standardized, objective manner.

***Speed.*** The throughput of image acquisition and analysis is important for at least three reasons. First, the faster the results are obtained, the sooner valid conclusions can be made and follow-on experiments planned. Second, when acquiring images of fluorescent signals, faster acquisition reduces signal fading. Third, multiple analysis assays can be applied to images to extract multiple features, or the same assay can be run over the data set using different 'stringencies' to extract different types of information. Modern HCA platforms can perform image acquisition and analysis in real time, rather than acquiring the images and then performing the analysis off-line. This greatly increases throughput speed.

***Accuracy/validity.*** As algorithms and hardware become more sophisticated, accuracy might increase to the point at which HCA becomes more accurate than even the human eye. For some applications HCA is already more accurate than methods such as biochemical assays[10]. Furthermore, because HCA is fast, it is possible to apply multiple different assays to the same image data set and determine the level of consistency in the results. When different assays generate the same trends, the conclusions are much stronger.

***Image processing.*** A major advantage of HCA is that powerful image-processing algorithms can be applied, to enhance signal-to-noise ratios and extract image features for more precise and accurate segmentation and analysis. Although it is desirable to analyse high-quality images that have been acquired at optimal settings, there are situations (for example, when a limit in the amount of tissue that is available precludes further experimentation) in which such image processing is needed. For example, morphology filters and other methods can remove artefacts before segmentation and measurement are carried out (FIG. 2).

***Other advantages.*** Because HCA is automated and fast, user-defined parameters can be manipulated and tested on image data sets to determine the appropriate level of

Box 1 | **Automated image acquisition and analysis**

Automation of both image acquisition and image analysis is the key to high-content analysis (HCA). The slowest step in image acquisition is autofocusing. The focus range must be wide enough to accommodate factors such as microplate curvature. However, modern platforms that use glass bottom plates have largely overcome such limitations, and so image acquisition is now fast. Image analysis is also fast and so can be carried out in real time as the images are acquired. Most platforms allow a range of magnifications.

Automated image analysis aims to identify and quantify specific aspects of images with as little human intervention as possible, and there are a number of packages that are free-standing or are part of HCA platforms[73]. Image filtering or noise reduction to remove imaging or staining artefacts is often the initial step (FIG. 2). Segmentation — selecting specific aspects of the image for further analysis (by assigning pixels to objects) — is the next step. This can be achieved by various methods, including intensity-thresholding methods, marker-based methods, watershed segmentation methods and edge-detection methods[73]. The cells can then be divided into subsets by classifiers (such as intensity, size or texture) and then measurements of specific cells can be taken. Many image-analysis programs have significant programing already installed for a range of cell-analysis applications, and some of these can be applied to acquired images in real time, which greatly increases the throughput.

Once the program has quantified and logged the image data, statistical analysis and higher-level interpretation of the results can be performed on the basis of knowledge of the experimental variables and information derived from other sources, such as public databases. For most academic applications this pathway of higher-level analysis is sufficient. However, by interfacing with image-mining software (such as Acuity Express, SpotFire and others), there is also the potential for automating this higher-level interpretation of image analyses. To achieve this, databases that hold details of the experimental procedures as well as the image-analysis results and public database information need to be developed[74]. This bioinformatics approach will be useful in computational modelling of brain cell biology[75].

**Glossary**

**Automated image analysis**
The use of automation to perform all of the functions of image analysis, including segmentation and data logging into spreadsheets.

**Automated microscopy**
The use of automation to control a microscope for image acquisition and storage.

**High-content analysis**
(HCA). A method for analysing complex cellular information (for example, signal intensity and localization) and morphology using automated microscopy and automated image analysis.

**High-content screening**
Another term for high-content analysis, but one that is generally used to describe high-content analysis that is performed at high throughput for screening purposes.

**Image filtering**
Removing noise from images.

**Image mining**
Using software to interface between analysed images and public databases, in order to extract higher-level information (for example, gene function and drug target).

**Lab-on-a-chip technology**
Miniaturized laboratory hardware that enables quicker and more resource-efficient experimentation and testing.

**Multiplexed assay**
An assay that extracts more than one piece of information from a data set. Many HCA assays are multiplexed. For example, an assay might count live cells, apoptotic cells and necrotic cells from the same well of a microplate by using different fluorescent channels to identify stages of cell survival and death.

**Morphology filters**
Mathematical operations for extracting information from images on the basis of size, shape and texture.

**Segmentation**
The isolation of a particular feature in an image — for example, a cell body if one is counting cells or a neurite if one is analysing outgrowth.

**Tissue microarray**
A method for patterning multiple samples of tissue onto individual microscope slides to standardize and automate tissue processing (for example, immunohistochemistry), image acquisition and image analysis.

stringency for these data sets. Furthermore, because HCA can integrate information from a number of channels (such as bright-field and fluorescence), more than one cell attribute can be measured in each image. This multiplexing allows for much more sophistication in data and image analysis and interpretation and generates much 'richer' cellular information than manual methods (FIG. 3). HCA also extracts information from whole cells, allowing biological context and relevance to be preserved, something that is not possible with biochemical methods. HCA thus provides a fast, accurate and objective way to quantify complex information from cells and tissues, contrasting with manual methods, which are generally laborious, slow and subjective. Furthermore, automated image acquisition and analysis might detect small but real differences that would elude a human observer.

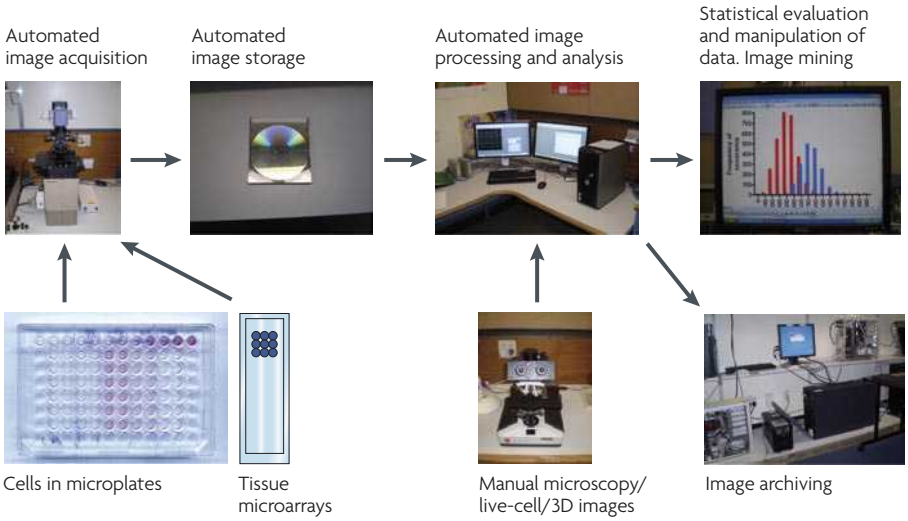


Figure 1 | **Steps in high-content analysis (HCA).** Images from cells in microplates (containing adherent cells or model organisms such as *Caenorhabditis elegans*) or tissue microarrays are acquired using automated microscopy (automated image acquisition). Robotized systems, which feature automated cell harvesting, immunocytochemistry, plate handling and image storage, can be used. The images then undergo appropriate automated image processing and analysis. This step can be applied in real time as the images are acquired (by modern HCA machines) or can be done off-line after image acquisition is complete (by older machines). Statistical evaluation and interpretation of image data sets is then completed: this might also be automated using image-mining software. Images can be archived for future use, for verification or for intellectual property and data management. Images that have been acquired manually from standard microscopes can also be processed and analysed automatically, as can live-cell images and three-dimensional (3D) images.

## HCA in neuroscience research

HCA has great potential to help unravel the brain's complexity at the cellular and sub-cellular levels. HCA is applicable to studies of normal and pathological brain function and can also be used as a high-throughput tool to screen therapies for brain disorders. Specific examples of the use of HCA in neuroscience are described below.

***Neurite outgrowth.*** Perhaps the most obvious application of HCA in neuroscience is to quantify the morphological complexity of axons and dendrites. Manual tracing software can accomplish this task, but it is laborious, slow and subjective. The advantages of high-throughput analysis of neurite outgrowth are speed and objectivity. Automated systems can measure many different aspects of neurites, including the amount of outgrowth (per image or per cell in experiments in which neurons are fixed at different time points), the amount of outgrowth occurring in the same cells over time (in live-cell time-lapse series images), the number of processes emerging from each cell, cross points from adjacent neurites, and their branching. Dendritic spines can also be measured. These measures of dendritic complexity are particularly important, as they are thought to be modified under both physiological and pathological conditions.

A recent study[11] used the Discovery-1 automated microscope with Metamorph image-analysis software to quantify neurite outgrowth in primary rat cortical cultures treated with amyloid-β, the protein that accumulates in the brains of patients with Alzheimer's disease. Although neuron number was not affected by amyloid-β, there was a large reduction in the outgrowth of neurites. Given that changes in neurites and synapses are thought to be causal events in the early stages of neurodegenerative diseases, this type of analysis might provide important information on the cause and potential treatment of more-subtle amyloid-β-mediated effects on neurons. This study highlights the power of the HCA approach: subtle changes in neuronal processes can be quantified in the absence of any overt cell death. Another example of the use of HCA to study neurite outgrowth comes from a study[12] that used Cellomics' ArrayScan II platform to automatically acquire and analyse images of human neural precursors in order to identify molecules with an effect on neuronal differentiation.

Both of these examples of the use of HCA to quantify neurites would have been difficult to perform using manual neurite-tracing methods because of the large numbers of images analysed. For this application, speed is the major advantage of the HCA approach. Another advantage is that many different aspects of neurites can be quantified simultaneously. Perhaps the greatest disadvantage of the automated neurite-outgrowth approaches is that they are not particularly adept at distinguishing the neurites of different neurons when they are highly intermeshed (as is common for neurons in the brain and also for those grown *in vitro*). There are HCA algorithms that can perform this separation and assign neurites to particular cells, but whether they can do this more accurately than a human is not clear. Because of this possible limitation, it would be preferable to measure dendritic morphology in neurons that do not overlap — this might be achieved by using micro-fabricated chips that anatomically separate neurons. Of course, the disadvantage of this approach is that communication between neurons might be artificially hindered and might therefore no longer represent the *in vivo* situation.

Many other automated methods for quantifying neurites have been developed. One group[13] developed an automated technique for quantifying changes in neuronal morphology and connectivity in time-lapse images of cultured neurons. They measured neurite attributes such as splitting, merging, growth and shrinkage at high throughput. Another group[14] developed an automated system for quantifying axonal growth in time-series images that performed as well as a human but at much greater throughput. Many other automated neurite-outgrowth assays have also been developed, validated

and shown to be accurate and fast tools for quantifying neurites[15–17].

Automated neurite-outgrowth assays can also be applied to cells in tissue sections. Ideally, such studies should use a nuclear stain or a neuron-specific stain, such as Neuronal N, to more precisely segment cell bodies from neurites (that is, they should use two separate labels). However, sometimes only one neuron label is available. In such instances, HCA algorithms can still be used to detect and quantify neuritic parameters (FIG. 4), but accuracy might be reduced. Furthermore, neurite-outgrowth assays can be applied to glial cells, which might change their morphology in response to drugs and in disease states (FIG. 4). For images in which there are few or no discernible cell bodies, high-throughput assays that measure the total length of the neurites can also be developed (see Supplementary information S1 (movie)).

***Neurogenesis, synaptogenesis and capillary formation.*** HCA has many applications in neurogenesis research[18]. In studies of neurogenesis, neurons are identified by a number of characteristics, such as dendritic processes (detected non-specifically with simple cell stains or specifically with antibodies against microtubule-associated protein 2 (MAP2)), expression of neuron-specific markers and neurophysiological responsiveness (such as conduction of appropriate electrical potentials). These 'neurons' and their processes can be counted manually by 'blinded' observers, but these approaches tend to be subjective and laborious. The advantages of using HCA for this type of analysis are its speed and its ability to measure degrees of neurite outgrowth. When HCA is combined with electrical testing of putative neurons, the conclusions as to whether the tested cells are indeed neurons are much stronger. Therefore, HCA assays that quantify neurite outgrowth and synaptogenesis are clearly relevant to neurogenesis studies, as are assays for DNA synthesis.

Researchers[19] have developed software that can automatically determine the lineage of a cell from time-series images of neural progenitors by following individual cells through numerous divisions and frames. Unlabelled cells are initially segmented by thresholding (on the basis of their intensity), and then a feature vector is constructed for each on the basis of parameters such as area, orientation, axis length and centroid coordinates. Using this information, a model is constructed that determines the likelihood (mathematically represented) of the cells' behaviour (movement, division, death, *et cetera*) in the next frame by following each cell and determining how it changes over time.

HCA software has also been used to quantify activity-dependent synapse formation in the hippocampus[20]. This enabled the authors to study the effects of mutations in the gene that encodes the synaptic-vesicle protein synaptophysin on synapse formation. Because synaptic contacts are very small, this type of analysis is laborious when performed manually. It takes many minutes per image to manually count the contacts, so it would be a major task to analyse hundreds or even thousands of images. HCA can perform such analyses in less than 1 second per image, making it ideal for such applications. The Metamorph HCA software also has an application for quantifying capillaries that has been successfully used to measure, at high throughput, cortical neovascularization after brain injury[21].

***Cell counting.*** A simple use for HCA assays is counting cells. Manual cell counting has been the method of necessity, rather than choice (as many graduate students would probably agree), for many years. It is subjective, slow and laborious. By contrast, HCA can perform cell counts objectively and at high speed. When cells have a simple rounded morphology, this type of analysis is straightforward. For example, Metamorph uses the 'Count nuclei' or 'Find spots' application to quickly and accurately count this type of material on the basis of cell size and stain intensity. However, brain cells have more complex morphologies, which make them much more difficult to segment and count. Furthermore, counting such cells in mixed cultures or in tissue sections in which there are a number of different cell types precludes the use of simple nuclear markers such as Hoechst stain or biochemical stains such as Alamar blue. Recently an automated high-throughput method for quantifying brain-cell numbers in mixed primary cultures was developed[22]. This assay allowed glial fibrillary acidic protein (GFAP)-positive astrocytes and CD45-positive microglia

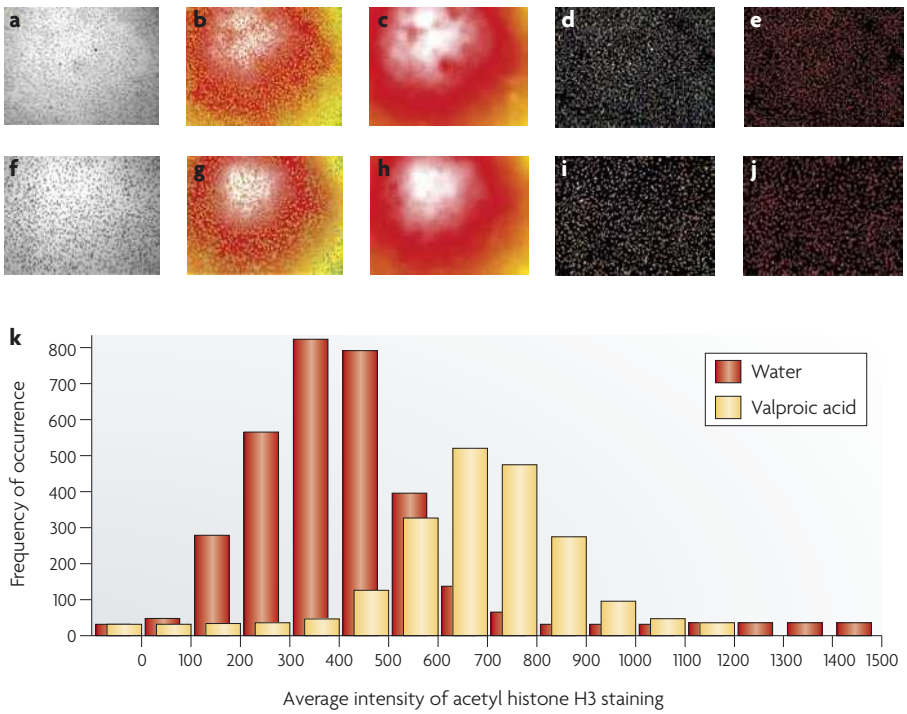


Figure 2 | **An example of image processing in high-content analysis (HCA).** Human brain cells, derived from temporal lobectomy tissue[76], were treated with either water (**a**–**e**) or 1 mM valproic acid (**f**–**j**) and fixed 24 hours later. Immunocytochemistry was then performed on the cells to detect acetylated histone H3. Brightfield images (**a**,**f**) were acquired using the Discovery-1 automated microscope. Converting the brightfield images into pseudocolour (**b**,**g**) reveals a major imaging artefact in each image. To reduce the influence of this artefact on the subsequent analysis, a background-correction image is generated for each brightfield image (**c**,**h**) and is then subtracted from its brightfield image to generate a corrected image (**d**,**i**). This corrected image is then processed (**e**,**j**) with the 'Count nuclei' application of Metamorph to extract staining intensity, which is graphed as a frequency distribution (**k**).

to be identified from either brightfield or fluorescent images. Because of the nature of the images, the assay required the incorporation of a number of morphology filters that enhanced cell contrast and removed debris, along with an integrated morphology analysis and configurations of object standards that specified cell size. The assay was tested against a range of image data sets and performed extremely well, as determined by manual counts. Furthermore, the assay was quick (taking just 1.82 seconds per image), making it a powerful tool for studying brain cells both in tissue culture and in tissue sections[22].

***Proliferation.*** Cell proliferation is a major area of research, particularly in the fields of glial cell biology and neurogenesis, in which the incorporation of compounds such as bromodeoxyuridine is used to label cells that are undergoing DNA synthesis[23]. HCA is ideal for quantifying the incorporation of stains such as bromodeoxyuridine when they are imaged either fluorescently or under transmitted light. For example, the Find spots application in Metamorph has been used to count both bromodeoxyuridine-positive dividing cells and total cells from transmitted-light images of C6 glioma cells[24], microglia and macrophages[25] quickly and accurately. Again, this type of counting is very slow, laborious and subjective when it is performed manually. Furthermore, when performing such studies in mixed cultures or in tissue sections, there is often a need to determine which cell types are incorporating the DNA-synthesis marker. HCA analysis algorithms can perform this more-complex analysis at high throughput. Finally, more recently neurogenesis researchers have developed techniques using a range of thymidine analogues (with different fluorescent tags) that enable the study of the proliferative history of neural stem cells *in vivo* and *in vitro*[26]. HCA analysis is ideally placed to quantify data from these types of experiments using multiple labels.

***Inclusion formation.*** Many neurodegenerative diseases, such as Huntington's disease, Alzheimer's disease, amyotrophic lateral sclerosis and Parkinson's disease, involve protein misfolding and aggregation[27], and studies of the mechanisms of inclusion formation and resolution are widely undertaken. However, most *in vitro* studies measure inclusion formation either by manual counting (which is slow, subjective and laborious) or biochemically (without cellular resolution). A high-throughput method for quantifying mutant-huntingtin (polyglutamine (polyQ) huntingtin) inclusion formation in PC12 cells was recently developed[28]. Four assays with differing throughputs and applications were developed, compared and validated (with manual counting and filter-retardation assays) (FIG. 5). For measuring the number and size of mutant huntingtin aggregates without reference to cell number, the polyQ assay was the quickest (taking 0.31 seconds per image), but to measure aggregates associated with cells, the 'Cell scoring' application in Metamorph was the most accurate. Combining such cell-based inclusion assays with read-outs for apoptosis might provide important insights into the role of aggregate formation in cell death processes. High-throughput automated image-analysis tools for detecting and counting amyloid-β-positive plaques and tau-positive tangles in sections of Alzheimer's disease brain tissue have also been developed and validated[29,30].

A recent study[31] used HCA to measure autophagosomes in a 96-well plate format with the ArrayScan HCS 4.0 Reader and Spot Detector BioApplication. Using this analysis, compounds that regulated autophagy and that might be useful in treating human diseases that involve misfolded proteins were identified. Similarly, an ArrayScan HCS reader was used to quantify, at high throughput, proteasomal activity in a proteasome-sensor cell line, to identify novel proteasome-inhibiting substances[32]. These HCA assays will be important tools for neuroscientists who are interested in the biology and treatment of protein-misfolding brain disorders. Their advantages over manual methods are their speed, accuracy and objectivity. They also have some advantages over biochemical methods, such as filter retardation assays[33], because they maintain the cellular context of the experiment and can therefore be used to study other aspects of cell biochemistry simultaneously. However, chemical methods that determine the actual physical structure of the aggregate[34] are better placed to unravel mechanistic aspects of how drugs might prevent aggregate formation. Combining these HCA methods with chemical methods provides a powerful platform for studying protein-misfolding disorders.

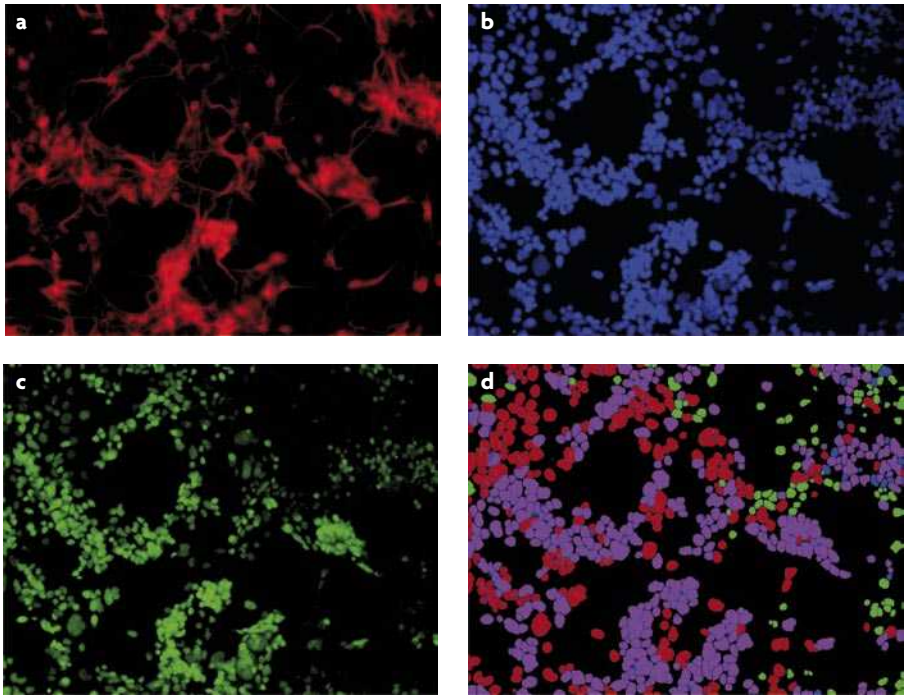


Figure 3 | **An example of a multiplexed assay.** P19 neurons were labelled with: an antibody against microtubule-associated protein 2 (MAP2) to detect dendrites (**a**), Hoechst to detect nuclei (**b**), and an antibody against activating transcription factor 2 (ATF2, a transcription factor that might be involved in neuronal differentiation[77]) (**c**). This particular experiment aimed to determine the proportion of MAP2-positive nerve cells that co-express ATF2. The three labels were processed using the 'Cell health' application in Metamorph. The results image (**d**) shows the combination of the three labels. The green overlay shows Hoechst-positive cells that are negative for the other two labels, the blue overlay shows Hoechst-positive cells that are MAP2-positive but ATF2-negative, the purple overlay shows triple-labelled cells and the red overlay shows Hoechst-positive cells that are ATF2-positive but MAP2-negative.

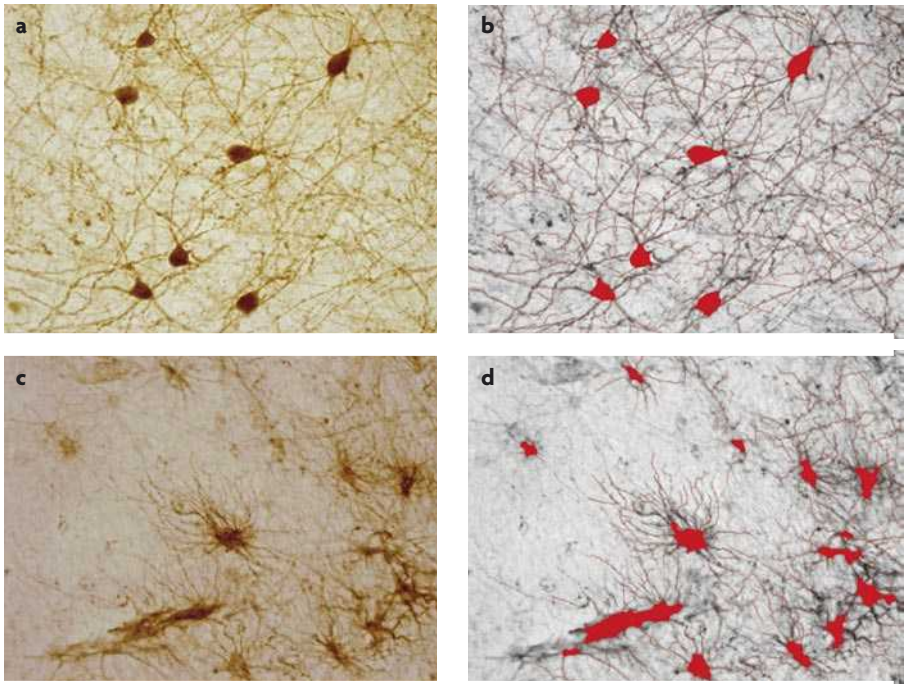


Figure 4 | **Measuring neurite outgrowth on tissue sections. a** | Parvalbumin-positive interneurons in the human temporal gyrus. **b** | The neurites and cell bodies of the neurons shown in **a** were segmented using the 'Neurite outgrowth' algorithm of Metamorph. **c** | Glial fibrillary acidic protein (GFAP)-positive astrocytes from the human temporal gyrus. **d** | The 'neurites' and cell bodies of the astrocytes shown in **c** were segmented using the Neurite outgrowth algorithm of Metamorph. The images shown in **a**–**d** were acquired manually using a Leica microscope at ×400 magnification.

***Apoptosis.*** Apoptosis is a feature of both the developing nervous system and adult brain disorders, and it is an important endpoint in many neuroscience contexts[35]. Cleaved caspase 3 immunocytochemistry, a marker of apoptosis, has been measured using an HCA assay in microglia[25]. A great advantage of using intact-cell-based methods to measure apoptosis is that other aspects of cell biochemistry can also be monitored. For example, one study[36] used a Cellomics platform to study activation of caspase 3, nuclear condensation (by staining with Hoechst) and cell viability (by staining with YoPro 1) in etoposide- and staurosporine-treated human NTera-2 cells. This multiplexed assay provided a powerful approach to study caspase inhibitors. The ability of HCA to perform multiplex assays again contrasts with manual methods of analysis, which are subjective and laborious.

Many new high-throughput biochemical methods of analysing apoptosis in cells and tissues are now available[37], and these are generally much cheaper (and easier) options than HCA. The disadvantage of these biochemical methods is that there is no cellular resolution. When there are multiple cell types in a tissue or in an *in vitro* experiment, these methods can still show evidence of apoptosis but cannot determine which cells are involved. By contrast, HCA approaches allow cellular resolution. Thus, the approach taken will depend to a large extent on the objectives of the experiment.

***Cell migration.*** Microglia and astrocytes in the brain migrate towards injuries and also towards 'lesions' such as amyloid plaques. Measuring migration *in vitro* is a widely used method to study glial cells. A high-throughput method has been developed to measure the migration of human astrocytes and glioma cells in two-dimensional scratch motility assays in 96-well plate formats[24,38]. Coomassie blue staining was used to image the cells, and brightfield images were acquired at ×20 magnification. The images were then processed using an 'open' morphology filter and thresholded for 'light objects' to segment and measure the scratch area (FIG. 6). This accurate and quick assay was sensitive enough to determine the effects of a MAPK/ERK kinase (MEK) blocker on the migration of astrocytes and glioma cells[24,38]. Another group[39] have developed an even-higher-throughput image-based cell-migration HCA assay using Metamorph software in a 384-well plate format, and others[40] have used an ArrayScan platform to measure human neural stem cell migration. These are powerful tools for studies of cell migration that could also be applied to the study of the migration of developing neurons and glia.

***Gap-junction quantification.*** Gap junctions are important mediators of cellular communication in the nervous system, and are important in glial scar formation[41] and neocortical radial migration[42]. An HCA-based assay has been developed that can measure gap-junction communication by imaging the movement of a fluorescent calcein dye using the ArrayScan II system[43]. This method was used to screen a large array of molecules that modify gap junctions. The anatomical resolution provided by HCA is ideal for studies of gap-junction function.

***Signal transduction.*** One of the most promising applications for HCA is in the signal-transduction field. Signal transduction involves temporal and spatial changes in signal strength and localization, both of which can be quantified by HCA.

Many neurotransmitter receptors are coupled through G proteins to their signal-transduction cascades. A common use of HCA assays in the biotechnology and pharmaceutical industries is in G-protein-coupled receptor (GPCR) studies. When agonists bind to these receptors they can induce receptor internalization, which can be measured with a number of HCA assays[3,44]. Recently a new HCA assay for studying internalization of the CB1 cannabinoid receptor was developed[45]. HA (hemagglutinin)-tagged CB1 receptors were transfected into HEK293 cells, and receptor internalization was imaged using antibodies against HA that are internalized with the receptor. This method has been compared with 'granularity' methods of receptor internalization (which segment and count granules/clusters of internalized receptors), and the two methods give comparable results. These HCA assays for studying GPCRs will be extremely useful for neuroscience applications because they use non-radioactive imaging methods that give high subcellular resolution and allow the compartmental complexity of receptors to be studied. When cellular resolution is not required, other ultra-high-throughput methods of analysis, such as cyclic AMP assays, would be preferred[46].

Imaging studies of β-arrestins, proteins that form complexes with GPCRs, can also provide important information regarding receptor signalling, and help to

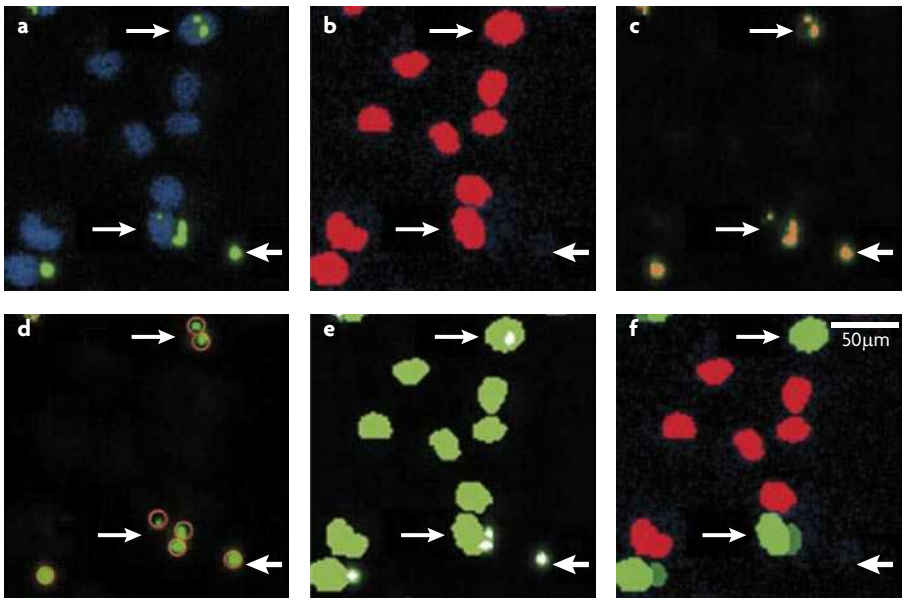


Figure 5 | **Measuring polyglutamine inclusions in cell culture with high-content analysis (HCA).** PC12 cells were transfected with a 97-repeat polyglutamine (polyQ) construct[33]. Images from transfected cells were acquired using the Discovery-1 automated fluorescence microscope from 96-well plates, and four separate analysis methods were used to quantify inclusions using Metamorph image-analysis software. **a** | Hoechst-positive nuclei (shown in blue), some of which (those indicated by the small arrows) are associated with inclusions (shown in green). The larger arrow shows an inclusion without a cell nucleus, suggesting that the cell that contained this inclusion had died. **b** | Segmentation (red overlay) of the nuclei shown in **a** using the 'Count nuclei' application in Metamorph. **c** | The polyQ assay segments inclusions on the basis of signal intensity (orange overlay). To obtain a total aggregate count, all of the thresholded objects are divided by a user-defined aggregate size. **d** | The 'Find spots' application in Metamorph uses user-defined inclusion-size and intensity parameters to identify and count inclusions. **e** | The 'Granularity' application of Metamorph (which is mainly used for studying receptor internalization) segments and counts granules (inclusions, shown as white objects) as well as cells (shown as green objects) with user-defined parameters. **f** | The 'Cell scoring' application of Metamorph counts nuclei that are positive (shown in light green) or negative (shown in red) for inclusions (shown in dark green). The advantages of this method are that it only counts inclusions that are associated with cells and that it automatically logs into spreadsheets the percentage of inclusion-positive and -negative cells. The disadvantages of this method are that it does not count all of the inclusions and that it requires more computer processing time than the PolyQ or Find spots assays. Please note that all of these steps are automated. Figure reproduced, with permission, from REF. 33 © (2008) Elsevier Science.

identify orphan GPCRs[47–49]. For example, translocation of green fluorescent protein (GFP)-tagged arrestin has been used to test for molecules that bind to GPCRs[48]. Cells were co-transfected with the GFP–arrestin and with the GPCR of interest and were then exposed to an agonist which caused translocation of the GFP–arrestin from the cytosol to the membrane. This powerful system can be used in a high-content platform for screening drugs against GPCRs. The assay can also be multiplexed to provide even more information[50].

Another key measure of signal transduction is the accumulation of intracellular $Ca^{2+}$ in response to neurotransmitters and hormones. HCA can be used to measure the extent and the kinetics of cellular $Ca^{2+}$ responses to various ligands[12,51,52]. For example, BD Pathway's HT Bioimager was used to perform kinetic analyses of $Ca^{2+}$ signalling in neural cells in response to various agents[12,52]. The HT Bioimager incorporates liquid-handling features for compound addition, making it ideal for measuring rapid-onset kinetic changes, which are a feature of $Ca^{2+}$ responses.

Transcription factors are important signalling molecules that are widely studied in neuroscience. Some transcription factors, such as nuclear factor-κB (NFκB), are activated by being translocated from the cytosol to the nucleus. Others, such as cAMP-responsive-element-binding protein (CREB), are expressed at high levels in the nucleus and are activated by phosphorylation, or are expressed at low levels basally but are rapidly induced and accumulate in the nucleus after certain types of stimulation (such as by c-FOS). HCA can quantify all three of these types of transcription factor processes. For example, the Find spots application in Metamorph was used to measure the expression of the inducible transcription factor KROX24 in Neuro2A cells in response to application of CB1 cannabinoid receptor agonists and antagonists[53]. This assay was validated using northern and western blotting and provided an excellent cell-based measure of transcription factor activation. HCA can also be used to measure transcription factor translocation (see Supplementary information S2 (figure)) and phosphorylation[54]. Automated methods for determining the subcellular localization of proteins have also been developed and might also be relevant to neuroscience applications[55].

All of the examples of HCA discussed so far have used platforms for adherent cells. Amnis Corporation has developed ImageStream, which brings together flow cytometry, fluorescence microscopy and high-throughput image analysis and allows HCA to be carried out on cells (and dissociated tissues) in suspension. This has been used in a number of applications[56], including the quantification of nuclear translocation of NFκB[57]. This technology might also be useful in certain neuroscience applications in which brain cells are grown in suspension.

## Future trends and applications

***Tissue microarrays.*** In addition to fixed cells and images acquired from time-lapse studies and three-dimensional imaging, HCA can also be applied to brain tissue[7]. To fully realize the potential of this approach, the image-acquisition and image-analysis stages should be automated. This is important for both throughput and, perhaps more importantly, standardization. Tissue microarrays are becoming important in drug discovery and target validation[58,59], and brain-tissue microarrays have been developed[60,61]. Software tools that have been developed for cell-based HCA can also be used on brain-tissue microarrays, and others have been specifically developed for tissue microarrays[62,63]. Tissue microarrays can be used for immunohistochemical and fluorescence *in situ* hybridization analysis.

The application of HCA to tissue microarrays will allow many aspects of brain-cell morphology and chemistry to be studied and quantified in a standardized, high-throughput and objective manner. For example, although there is a belief that changes in dendritic arborization occur early in neurodegenerative disorders, the ability

to quantify branching in tissue sections will allow a much more quantitative approach. As an example of this potential, we are currently engaged on a project that is investigating neurite outgrowth in various cortical regions from neurologically normal and Huntington's disease post-mortem brains. These studies are currently being performed on whole sections, and the use of tissue microarrays will greatly augment them.

***Lab-on-a-chip formats.*** Recent advances in microfluidics and lithography have realized the possibility of developing lab-on-a-chip technologies for biomedical research and drug discovery[64,65]. HCA of such chips has been accomplished[66–68], and lab-on-a-chip methods have also been applied to the nervous system, for which they provide powerful high-thoughput platforms[69].

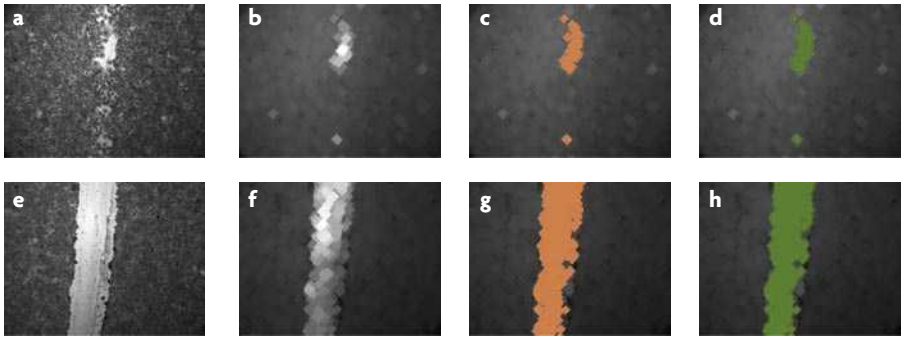


Figure 6 | **Measuring astrocyte migration in cell culture with high-content analysis (HCA).** Confluent monolayers of human astrocytes grown in 96-well plates were scratched with a pipette tip[38]. Five days later (**a**–**d**), or immediately after (**e**–**h**), cells were fixed and stained with Coomassie blue, which stains the entire cell and provides good contrast for imaging and segmenting cell-free regions (that is, the scratch). Images were acquired using transmitted light on a Discovery-1 automated microscope and then processed and quantified using Metamorph image-analysis software. The objective of the assay was to measure the 'gap area' (the white unstained region), which provides a measure of the migration of the cells (a small gap area equates to rapid migration). An 'open' morphology filter that filters pixel grey values with user-defined size parameters was used to initially process the images (**b**,**f**). This step was carried out to reduce false positives by reducing the impact of small 'gaps' outside the scratch area. The images were then thresholded for light objects (**c**,**g**; orange overlay) to segment out the region of interest (in this case the gap area). Image morphology analysis was then used to measure the thresholded area (**d**,**h**; the gap area), which was automatically logged into spreadsheets. Please note that all of the image processing, segmentation-analysis and logging steps are automated using Journals (macros for writing short programs). Figure reproduced, with permission, from REF. 38 © (2007) Elsevier/North-Holland Biomedical Press.

## Running an academic HCA laboratory

Over the past 5 years of running an academic HCA laboratory, a number of lessons have been learnt. The human component of HCA is crucial, and the user must have a good understanding of the biology that they are measuring, as well as of the image-analysis program. There are also many important issues of data storage, archiving and analysis that need to be addressed. One of the most interesting aspects of HCA is the development, validation and publication of in-house assays. Often applications that come as part of the software package can be used for alternative purposes. This type of flexibility is powerful, especially in a complex imaging field such as neuroscience.

Many academic laboratories cannot afford high-throughput automated-microscopy platforms, or automation might not be possible in particular applications. In these circumstances images can be acquired using standard manual microscopy methods and analysed by HCA software at high throughput. It is important that image acquisition is standardized as far as possible. A recent article[70] provides an excellent review of methods for the automated analysis of such images. My laboratory has begun to use HCA methods on images acquired in this way from human brain specimens to quantify various aspects of brain anatomy[7] (also see Supplementary information S3 (movie) and S4 (movie)).

What are the advantages and disadvantages of the HCA approach compared with manual and/or biochemical methods for an academic laboratory? The greatest disadvantage of HCA is the relatively high cost of setting up and maintaining the automated microscopy platforms. However, having automated image-analysis software on off-line computers means that, even if the automated microscope is being repaired, images acquired on manual microscopes or archived images can still be analysed. Another disadvantage of HCA compared with biochemical methods of analysis[71] is the amount of human input that is required for HCA (although this is still much less than is required for manual image-analysis methods). However, HCA is superior to other methods of analysis for quantifying cell morphology, subcellular protein localization or more than one attribute simultaneously. If only signal intensity is being quantified in a homogeneous cell population, other simpler and much cheaper methods are preferable. For example, cell-based enzyme-linked immunosorbent assay (ELISA) performed extremely well in quantifying the induction of the KROX24 transcription factor by muscarinic receptor agonists in homogeneous human neuroblastoma cells[71]. There have been major advances in HCA hardware, and if costs can be contained then this aspect of HCA is well advanced. However, there is much more room for development in automated image-analysis software design — to increase accuracy and throughput and to further reduce human involvement. Associated with this is the further development of image-mining tools for higher-level interpretation of HCA outputs. Neuroscientists, in collaboration with computer experts, could play an important part in these types of software developments.

## Conclusion

The preceding discussion illustrates the wide applicability of HCA to neuroscience. Many HCA assays are multiplexed, and so provide rich data sets that measure multiple aspects of cell biochemistry and morphology. In addition, HCA can be applied to images acquired from a range of modalities, including the use of automated microscopes, manual microscopes, live-cell images, three-dimensional images, tissue microarrays and lab-on-a-chip formats, as well as to MRI images of the whole human brain[72]. HCA has already proven to be a powerful technology for various neuroscience applications, and doubtless the wider neuroscience community will soon adopt it too. Cellular informatics software that links HCA platforms with public databases for image mining is also now becoming commercially available, or might be fostered in academic settings by collaborations between neuroscientists and computer scientists. This should further increase the usefulness of HCA to neuroscience, in particular for functional genomics studies and for drug discovery for brain disorders.

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1. Dove, A. Screening for content—the evolution of high throughput. *Nature Biotechnol.* **21**, 859–864 (2003).
2. Evans, J. G. & Matsudaira, P. Linking microscopy and high content screening in large-scale biomedical research. *Methods Mol. Biol.* **356**, 33–38 (2007).
3. Granas, C. *et al.* High content screening for G protein-coupled receptors using cell-based protein translocation assays. *Comb. Chem. High Throughput Screen.* **8**, 301–309 (2005).
4. Lang, P., Yeow, K., Nichols, A. & Scheer, A. Cellular imaging in drug discovery. *Nature Rev. Drug Discov.* **5**, 343–356 (2006).
5. Perlman, Z. E. *et al.* Multidimensional drug profiling by automated microscopy. *Science* **306**, 1194–1198 (2004).
6. Taylor, D. L., Woo, E. S. & Giuliano, K. A. Real-time molecular and cellular analysis: the new frontier of drug discovery. *Curr. Opin. Biotechnol.* **12**, 75–81 (2001).
7. Curtis, M. A. *et al.* Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. *Science* **315**, 1243–1249 (2007).
8. Burns, A. R. *et al.* High-throughput screening of small molecules for bioactivity and target identification in *Caenorhabditis elegans*. *Nature Protoc.* **1**, 1906–1914 (2006).
9. Vaisberg, E. A., Lenzi, D., Hansen, R. L., Keon, B. H. & Finer, J. T. An infrastructure for high-throughput microscopy: instrumentation, informatics, and integration. *Methods Enzymol.* **414**, 484–512 (2006).
10. Dragunow, M., Cameron, R., Narayan, P. & O'Carroll, S. Image-based high-throughput quantification of cellular fat accumulation. *J. Biomol. Screen.* **12**, 999–1005 (2007).
11. Hu, M. *et al.* High content screen microscopy analysis of $A\beta_{1-42}$-induced neurite outgrowth reduction in rat primary cortical neurons: neuroprotective effects of $\alpha 7$ neuronal nicotinic acetylcholine receptor ligands. *Brain Res.* **1151**, 227–235 (2007).
12. Richards, G. R. *et al.* A morphology- and kinetics-based cascade for human neural cell high content screening. *Assay Drug Dev. Technol.* **4**, 143–152 (2006).
13. Al-Kofahi, O., Radke, R. J., Roysam, B. & Banker, G. Automated semantic analysis of changes in image sequences of neurons in culture. *IEEE Trans. Biomed. Eng.* **53**, 1109–1123 (2006).
14. Keenan, T. M. *et al.* Automated identification of axonal growth cones in time-lapse image sequences. *J. Neurosci. Methods* **151**, 232–238 (2006).
15. Ramm, P. *et al.* Automated screening of neurite outgrowth. *J. Biomol. Screen.* **8**, 7–18 (2003).
16. Price, R. D., Oe, T., Yamaji, T. & Matsuoka, N. A simple, flexible, nonfluorescent system for the automated screening of neurite outgrowth. *J. Biomol. Screen.* **11**, 155–164 (2006).
17. Vallotton, P. *et al.* Automated analysis of neurite branching in cultured cortical neurons using HCA-Vision. *Cytometry A* **71**, 889–895 (2007).
18. Curtis, M. A., Faull, R. L. & Eriksson, P. S. The effect of neurodegenerative diseases on the subventricular zone. *Nature Rev. Neurosci.* **8**, 712–723 (2007).
19. Al-Kofahi, O. *et al.* Automated cell lineage construction: a rapid method to analyze clonal development established with murine neural progenitor cells. *Cell Cycle* **5**, 327–335 (2006).
20. Tarsa, L. & Goda, Y. Synaptophysin regulates activity-dependent synapse formation in cultured hippocampal neurons. *Proc. Natl Acad. Sci. USA* **99**, 1012–1016 (2002).
21. Svedin, P. *et al.* Delayed peripheral administration of a GPE analogue induces astrogliosis and angiogenesis and reduces inflammation and brain injury following hypoxia-ischemia in the neonatal rat. *Dev. Neurosci.* **29**, 393–402 (2007).
22. Narayan, P. J., Gibbons, H. M., Mee, E. W., Faull, R. L. & Dragunow, M. High throughput quantification of cells with complex morphology in mixed cultures. *J. Neurosci. Methods* **164**, 339–349 (2007).
23. Taupin, P. BrdU immunohistochemistry for studying adult neurogenesis: paradigms, pitfalls, limitations, and validation. *Brain Res. Rev.* **53**, 198–214 (2007).
24. Lind, C. R. *et al.* The mitogen-activated/extracellular signal-regulated kinase kinase 1/2 inhibitor U0126 induces glial fibrillary acidic protein expression and reduces the proliferation and migration of C6 glioma cells. *Neuroscience* **141**, 1925–1933 (2006).
25. Dragunow, M. *et al.* Valproic acid induces caspase 3-mediated apoptosis in microglial cells. *Neuroscience* **140**, 1149–1156 (2006).
26. Vega, C. J. & Peterson, D. A. Stem cell proliferative history in tissue revealed by temporal halogenated thymidine analog discrimination. *Nature Methods* **2**, 167–169 (2005).
27. Soto, C. Unfolding the role of protein misfolding in neurodegenerative diseases. *Nature Rev. Neurosci.* **4**, 49–60 (2003).
28. Scotter, E. L., Narayan, P., Glass, M. & Dragunow, M. High throughput quantification of mutant huntingtin aggregates. *J. Neurosci. Methods* **171**, 174–179 (2008).
29. Teboul, O. *et al.* A standardized method to automatically segment amyloid plaques in Congo Red stained sections from Alzheimer transgenic mice. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* **2007**, 5593–5596 (2007).
30. Chubb, C. *et al.* BioVision: an application for the automated image analysis of histological sections. *Neurobiol. Aging* **27**, 1462–1476 (2006).
31. Zhang, L. *et al.* Small molecule regulators of autophagy identified by an image-based high-throughput screen. *Proc. Natl Acad. Sci. USA* **104**, 19023–19028 (2007).
32. Rickardson, L., Wickstrom, M., Larsson, R. & Lovborg, H. Image-based screening for the identification of novel proteasome inhibitors. *J. Biomol. Screen.* **12**, 203–210 (2007).
33. Scotter, E. L., Narayan, P., Glass, M. & Dragunow, M. High throughput quantification of mutant huntingtin aggregates. *J. Neurosci. Methods* **171**, 174–179 (2008).
34. Lee, C. C., Walters, R. H. & Murphy, R. M. Reconsidering the mechanism of polyglutamine peptide aggregation. *Biochemistry* **46**, 12810–12820 (2007).
35. Bredesen, D. E. Key note lecture: toward a mechanistic taxonomy for cell death programs. *Stroke* **38**, 652–660 (2007).
36. Fennell, M., Chan, H. & Wood, A. Multiparameter measurement of caspase 3 activation and apoptotic cell death in NT2 neuronal precursor cells using high-content analysis. *J. Biomol. Screen.* **11**, 296–302 (2006).
37. Huang, R. *et al.* Characterization of diversity in toxicity mechanism using *in vitro* cytotoxicity assays in quantitative high throughput screening. *Chem. Res. Toxicol.* **21**, 659–667 (2008).
38. Lim, J. H. *et al.* Extracellular signal-regulated kinase involvement in human astrocyte migration. *Brain Res.* **1164**, 1–13 (2007).
39. Yarrow, J. C., Perlman, Z. E., Westwood, N. J. & Mitchison, T. J. A high-throughput cell migration assay using scratch wound healing, a comparison of image-based readout methods. *BMC Biotechnol.* **4**, 21 (2004).
40. Richards, G. R., Millard, R. M., Leveridge, M., Kerby, J. & Simpson, P. B. Quantitative assays of chemotaxis and chemokinesis for human neural cells. *Assay Drug Dev. Technol.* **2**, 465–472 (2004).
41. Seifert, G., Schilling, K. & Steinhauser, C. Astrocyte dysfunction in neurological disorders: a molecular perspective. *Nature Rev. Neurosci.* **7**, 194–206 (2006).
42. Elias, L. A., Wang, D. D. & Kriegstein, A. R. Gap junction adhesion is necessary for radial migration in the neocortex. *Nature* **448**, 901–907 (2007).
43. Li, Z. *et al.* Identification of gap junction blockers using automated fluorescence microscopy imaging. *J. Biomol. Screen.* **8**, 489–499 (2003).
44. Haasen, D., Schnapp, A., Valler, M. J. & Heilker, R. G protein-coupled receptor internalization assays in the high-content screening format. *Methods Enzymol.* **414**, 121–139 (2006).
45. Grimsey, N. L., Narayan, P. J., Dragunow, M. & Glass, M. Novel high-throughput assay for the quantitative assessment of receptor trafficking. *Clin. Exp. Pharmacol. Physiol.* 28 Jun 2008 (10.1111/j.1440-1681.2008.04991.x).
46. Weber, M. *et al.* Ultra-high-throughput screening for antagonists of a $G_i$-coupled receptor in a 2.2-microl 3,456-well plate format cyclicAMP assay. *Assay Drug Dev. Technol.* **5**, 117–125 (2007).
47. DeWire, S. M., Ahn, S., Lefkowitz, R. J. & Shenoy, S. K. β-arrestins and cell signaling. *Annu. Rev. Physiol.* **69**, 483–510 (2007).
48. Hudson, C. C., Oakley, R. H., Sjaastad, M. D. & Loomis, C. R. High-content screening of known G protein-coupled receptors by arrestin translocation. *Methods Enzymol.* **414**, 63–78 (2006).
49. Oakley, R. H., Hudson, C. C., Sjaastad, M. D. & Loomis, C. R. The ligand-independent translocation assay: an enabling technology for screening orphan G protein-coupled receptors by arrestin recruitment. *Methods Enzymol.* **414**, 50–63 (2006).
50. Ross, D. A. *et al.* Multiplexed assays by high-content imaging for assessment of GPCR activity. *J. Biomol. Screen.* **13**, 449–455 (2008).
51. DeBernardi, M. A. & Brooker, G. High-content kinetic calcium imaging in drug-sensitive and drug-resistant human breast cancer cells. *Methods Enzymol.* **414**, 317–335 (2006).
52. Richards, G. R., Jack, A. D., Platts, A. & Simpson, P. B. Measurement and analysis of calcium signaling in heterogeneous cell cultures. *Methods Enzymol.* **414**, 335–347 (2006).
53. Graham, E. S. *et al.* Induction of Krox-24 by endogenous cannabinoid type 1 receptors in Neuro2A cells is mediated by the MEK-ERK MAPK pathway and is suppressed by the phosphatidylinositol 3-kinase pathway. *J. Biol. Chem.* **281**, 29085–29095 (2006).
54. Tencza, S. B. & Sipe, M. A. Detection and classification of threat agents via high-content assays of mammalian cells. *J. Appl. Toxicol.* **24**, 371–377 (2004).
55. Chen, X. & Murphy, R. F. Automated interpretation of protein subcellular location patterns. *Int. Rev. Cytol.* **249**, 193–227 (2006).
56. George, T. C. *et al.* Distinguishing modes of cell death using the ImageStream multispectral imaging flow cytometer. *Cytometry A* **59**, 237–245 (2004).
57. George, T. C. *et al.* Quantitative measurement of nuclear translocation events using similarity analysis of multispectral cellular images obtained in flow. *J. Immunol. Methods* **311**, 117–129 (2006).
58. Sauter, G., Simon, R. & Hillan, K. Tissue microarrays in drug discovery. *Nature Rev. Drug Discov.* **2**, 962–972 (2003).
59. Braunschweig, T., Chung, J. Y. & Hewitt, S. M. Perspectives in tissue microarrays. *Comb. Chem. High Throughput Screen.* **7**, 575–585 (2004).
60. Martikainen, P., Louhelainen, A. M., Kauppinen, T. & Alafuzoff, I. Human brain tissue microarrays as a platform to investigate diseases of the nervous system. *Brain Res.* **1089**, 33–43 (2006).
61. Takei, H., Buckleair, L. W., Rivera, A. & Powell, S. Z. Brain tissue microarrays in neurodegenerative diseases: validation of methodology and immunohistochemical study of growth-associated protein-43 and calretinin. *Pathol. Int.* **57**, 775–783 (2007).
62. Liu, C. L. *et al.* Software tools for high-throughput analysis and archiving of immunohistochemistry staining data obtained with tissue microarrays. *Am. J. Pathol.* **161**, 1557–1565 (2002).
63. Camp, R. L., Chung, G. G. & Rimm, D. L. Automated subcellular localization and quantification of protein expression in tissue microarrays. *Nature Med.* **8**, 1323–1327 (2002).
64. Dittrich, P. S. & Manz, A. Lab-on-a-chip: microfluidics in drug discovery. *Nature Rev. Drug Discov.* **5**, 210–218 (2006).
65. El-Ali, J., Sorger, P. K. & Jensen, K. F. Cells on chips. *Nature* **442**, 403–411 (2006).
66. Ziauddin, J. & Sabatini, D. M. Microarrays of cells expressing defined cDNAs. *Nature* **411**, 107–110 (2001).
67. Erfle, H. *et al.* Reverse transfection on cell arrays for high content screening microscopy. *Nature Protoc.* **2**, 392–399 (2007).
68. Neumann, B. *et al.* High-throughput RNAi screening by time-lapse imaging of live human cells. *Nature Methods* **3**, 385–390 (2006).
69. Ionescu-Zanetti, C. *et al.* Mammalian electrophysiology on a microfluidic platform. *Proc. Natl Acad. Sci. USA* **102**, 9112–9117 (2005).
70. Theodosiou, Z. *et al.* Automated analysis of FISH and immunohistochemistry images: a review. *Cytometry A* **71**, 439–450 (2007).
71. Greenwood, J. M. & Dragunow, M. Muscarinic receptor-mediated phosphorylation of cyclic AMP response element binding protein in human neuroblastoma cells. *J. Neurochem.* **82**, 389–397 (2002).
72. Fischl, B. *et al.* Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* **33**, 341–355 (2002).

73. Wollman, R. & Stuurman, N. High throughput microscopy: from raw images to discoveries. *J. Cell Sci.* **120**, 3715–3722 (2007).
74. Berlage, T. Analyzing and mining image databases. *Drug Discov. Today* **10**, 795–802 (2005).
75. Sharan, R. & Ideker, T. Modeling cellular machinery through biological network comparison. *Nature Biotechnol.* **24**, 427–433 (2006).
76. Gibbons, H. M. *et al.* Cellular composition of human glial cultures from adult biopsy brain tissue. *J. Neurosci. Methods* **166**, 89–98 (2007).
77. Pearson, A. G., Curtis, M. A., Waldvogel, H. J., Faull, R. L. & Dragunow, M. Activating transcription factor 2 expression in the adult human brain: association with both neurodegeneration and neurogenesis. *Neuroscience* **133**, 437–451 (2005).

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**DATABASES**
**Entrez Gene:** http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
MAP2 | synaptophysin
**OMIM:** http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
Alzheimer's disease | amyotrophic lateral sclerosis | Huntington's disease | Parkinson's disease

**FURTHER INFORMATION**
**Mike Dragunow's homepage:** http://www.fmhs.auckland.ac.nz/sms/pharmacology/discovery1/

**SUPPLEMENTARY INFORMATION**
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OPINION

# Cognitive therapy versus medication for depression: treatment outcomes and neural mechanisms

*Robert J. DeRubeis, Greg J. Siegle and Steven D. Hollon*

Abstract | Depression is one of the most prevalent and debilitating of the psychiatric disorders. Studies have shown that cognitive therapy is as efficacious as antidepressant medication at treating depression, and it seems to reduce the risk of relapse even after its discontinuation. Cognitive therapy and antidepressant medication probably engage some similar neural mechanisms, as well as mechanisms that are distinctive to each. A precise specification of these mechanisms might one day be used to guide treatment selection and improve outcomes.

Major depressive disorder is a serious illness that in the United States alone is estimated to affect 13 to 14 million adults each year. The lifetime prevalence rate (16%) is even higher, with an estimated 32 to 35 million US residents expected to develop the disorder at some point during their lifetime. Not only can depression be debilitating on its own, it also has a high rate of co-morbidity with other mental illnesses. Indeed, nearly three-quarters of people who meet the criteria for depression at some point during their lifetime will also meet the criteria for another psychiatric disorder: approximately three-fifths will be co-morbid for one of the anxiety disorders, one-quarter for substance-use disorders and one-third for impulse-control disorders[1].

Substantial impairment in social and occupational functioning is also frequently observed in depressed individuals[2]. Not surprisingly, the economic burden of depression is enormous. Workplace-related costs in the United States have been estimated at more than 50 billion dollars annually[3]. Although most episodes of depression resolve without treatment, over one-quarter of all patients suffer from chronic depression, and the vast majority of those who do recover from a depressive episode will experience recurrences. Owing to its prevalence, its chronic and recurrent nature and its frequent co-morbity with other chronic illnesses — both as a contributing factor and as a consequence — depression is considered to be the condition that is most responsible for health decrements worldwide. It is therefore a global health priority to understand, prevent and treat depression[4].

## The nature of depression

Depression can be defined as both a syndrome and a disorder. As a syndrome it involves episodes of sadness, loss of interest, pessimism, negative beliefs about the self, decreased motivation, behavioural passivity, changes in sleep, appetite and sexual interest, and suicidal thoughts and impulses. As a disorder it comes in two forms. The unipolar type, which affects approximately 10% of men and 20% of women, includes only episodes of depression[1]. Heritability estimates for this unipolar type have ranged from approximately 25% in less-severe samples up to 50% in more-severe samples[5].

In the bipolar form, which is commonly known as manic depression, patients also (or exclusively) experience episodes of mania or hypomania that are in many ways the opposite of depression. Manic episodes are marked by euphoria or irritability, sleeplessness, grandiosity, recklessness and uncontrollable impulses that can lead to buying sprees and sexual promiscuity[6]. This Perspective concentrates on unipolar depression, as the phenomenology differs from bipolar depression, as do the medication and psychological treatments. In addition, more is known about the neural mechanisms that underlie unipolar disorder and its treatment, and it is unclear how these mechanisms are related to those of bipolar disorder.

## Current treatment practices

The goal of acute treatment for unipolar depression with antidepressant medication (ADM), the current standard of treatment, is generally to provide symptom relief. In this context, response is defined as a noticeable improvement, and remission is defined by the near absence of symptoms[7]. Even when remission is achieved, patients retain a high risk of relapse (that is, the return of symptoms within the same episode). For this reason, patients who have been treated to the point of remission with ADM are advised to continue treatment for at least 6 months (the period of greatest risk of relapse). Patients whose remission lasts for 6 months are said to have recovered. However, those who recover from depression with ADM but then discontinue the treatment have a risk of experiencing a new episode of depression (recurrence) that is 3 to 5 times the risk of a member of the general population experiencing a first episode of depression. Consequently, practice guidelines emphasize the benefits of maintaining recovered patients on ADMs indefinitely, especially for patients who have a history of recurrent (or chronic) depression.